Current Microbiology
https://doi.org/10.1007/s00284-018-1441-0

LETTER TO THE EDITOR

Complete Genome Sequence of *Pseudomonas Parafulva* PRS09-11288, a Biocontrol Strain Produces the Antibiotic Phenazine-1-carboxylic Acid

Yu Zhang[1] · Ping Chen[1] · Guoyou Ye[2] · Haiyan Lin[1,2] · Deyong Ren[1] · Longbiao Guo[1] · Bo Zhu[3,4] · Zhongwei Wang[1]

Received: 8 October 2017 / Accepted: 17 January 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Rhizoctonia solani is a plant pathogenic fungus, which can infect a wide range of economic crops including rice. In this case, biological control of this pathogen is one of the fundmental way to effectively control this pathogen. The *Pseudomonas parafulva* strain PRS09-11288 was isolated from rice rhizosphere and shows biocontrol ability against *R. solani*. Here, we analyzed the *P. parafulva* genome, which is ~4.7 Mb, with 4310 coding sequences, 76 tRNAs, and 7 rRNAs. Genome analysis identified a phenazine biosynthetic pathway, which can produce antibiotic phenazine-1-carboxylic acid (PCA). This compound is responsible for biocontrol ability against *R. solani* Kühn, which is one of the most serious fungus disease on rice. Analysis of the phenazine biosynthesis gene mutant, *ΔphzF*, which is very important in this pathway, confirmed the relationship between the pathway and PCA production using LC-MS profiles. The annotated full genome sequence of this strain sheds light on the role of *P. parafulva* PRS09-11288 as a biocontrol bacterium.

Background

Rhizoctonia solani Kühn, which can cause sheath blight of rice, is one of the most destructive rice disease in the world [11]. It has been reported that this pathogen can cause yield losses up to 50% under disease favoring conditions [9]. Due to its importance, it is strongly needed to find a way to control this pathogen effectively. However, breeding of the resistant rice plant is not very successful due to the lack of availability of resistant donor [4]. In addition, since *R. solani* is a soil-borne fungus disease [16], the application of fungicides in soil is not so effective to control this pathogen. In addition, misuse of fungicide may result in soil contamination. In this case, biocontrol using some bacterial strains is an alternative way to control the outbreak of this disease with little negative environmental consequences [2, 7].

Natural antibiotics produced by some bacteria are thought to give them some fitness and competitiveness advantages against other microorganisms in the environment [14]. These nature antibiotics include a large class of well-known secondary metabolites including phenazines, which are mainly from microorganisms such as *Pseudomonas* spp [12]. These phenazines include phenazine-1-carboxylic acid (PCA), which is a broad-spectrum antibiotic produced by many *Pseudomonas* spp. in the rhizosphere [14]. PCA has long been reported to be a good

Yu Zhang and Ping Chen have contributed equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-018-1441-0) contains supplementary material, which is available to authorized users.

✉ Bo Zhu
bzhu1981@sjtu.edu.cn

✉ Zhongwei Wang
390346815@qq.com

1 State Key Lab for Rice Biology, China National Rice Research Institute, Hangzhou 310006, People's Republic of China

2 Agricultural Genomes Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, People's Republic of China

3 School of Agriculture and Biology, Shanghai Jiao Tong University/Key Laboratory of Urban Agriculture by Ministry of Agriculture of China, Shanghai 200240, People's Republic of China

4 State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, People's Republic of China

Published online: 22 January 2018

biocontrol agent against many soil-borne plant pathogens [22]. Recent report suggested that these PCA produced *Pseudomonas* spp. has good antagonistic ability against wheat *R. solani* [15]. In 2003, Xie et al. isolated several *Pseudomonas* spp., including *P. parafulva* PRS09-11288, from rice seeds that were collected from Philippines [25]. This isolate showed good antagonistic potential against rice *R. solani*.

Recently, we detected the existence of phenazine biosynthesis gene *phzF*, which is a biomarker for Phz^+ pseudomonads [18]. This result suggested that the biocontrol ability of PRS09-11288 may be attributed to PCA production. The antagonistic mechanism of this strain is totally different from another sequenced biocontrol *P. parafulva* strain CRS01-1 [13]. To address this question, we selected this strain for whole-genome sequencing and genome sequence analysis.

DNA Extraction and Whole-Genome Sequencing

Single colony of PRS09-11288 was inoculated into 3 ml NB (Nutrient Broth, BD, USA) at 30 °C with 150 rpm vigorous shaking. 2 ml of culture broth was used to isolate the genomic DNA. DNA was extracted using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The quality of purified genomic DNA was tested by NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, MA, USA) and Qubit 2.0 fluorometer (Life Technologies, MA, USA), respectively. The genome of PRS09-11288 was sequenced with PacBio RS II platform. Around 600 Mb raw data was obtained with 100X average coverage.

Genome Assembly and Annotation

After quality control, genome assembly was de novo assembled using HGAP assembly protocol, which is available with the SMRT Analysis packages and accessed through the SMRT Analysis Portal version 2.1. Genome annotation was later done using RAST annotation system [17]. In addition, GO and COG programs were used to do further functional analysis of all annotated ORFs [3, 21]. Antibiotic and secondary metabolite analysis was done by searching against the antiSMASH and DoBISCUIT databases [6, 24]. The circular genome map of PRS09-11288 including all predicted ORFs with COG functional assignments, rRNA, tRNA, G+C content and GC skew information were generated using Circos [10], as shown in Fig. S1.

General Genome Sequence Property

The total size of the genome is 4,685,985 bp and has a GC content of 61.70%. A total of 4160 CDSs were predicted. Of these, 3075 could be assigned to a COG number. The most abundant COG category was “Amino acid transport and metabolism” (460 proteins) followed by “Signal transduction mechanisms” (457 proteins), “Transcription” (340 proteins), and “Energy production and conversion” (257 proteins). In addition, 83 RNAs including rRNA and tRNA were identified. All the genomic information was shown in Table 1.

Phenazine Biosynthetic Pathway in PRS09-11288

To identify the genes related to phenazine biosynthetic pathway, the associated genes from model genome *Pseudomonas aeruginosa* PAO1 was used to search against PRS09-11288. This process identified a phenazine biosynthetic pathway

Table 1 Genomic features of *P. parafulva* PRS09-11288

Feature	Value
Genome size	4,685,985 bp
DNA coding	4,118,721 bp
DNA G+C	61.71%
Chromosome	1
Total genes	4310
Protein coding genes	4160
RNA genes	83
Genes with functional prediction	3499
Genes assigned to COGs	3075
Genes with Pfam domains	3577
Genes with signal peptides	413
Genes with transmembrane helices	908
Genes related to secondary metabolism	899
Genes related to antibiotic resistance	281

Table 2 Proteins with similarity to the phenazine biosynthetic pathway in *P. parafulva* PRS09-11288

Accession number	Gene name	Description
B2J77_14050	*phzA*	Phenazine biosynthesis protein
B2J77_14055	*phzB*	Phenazine biosynthesis protein
B2J77_14060	*phzD*	Isochorismatase
B2J77_14065	*phzE*	Anthranilate synthase
B2J77_14070	*phzG*	Phenazine biosynthesis protein
B2J77_14075	*phzF*	2,3-Dihydro-3-hydroxyanthranilate isomerase
B2J77_14080	*phzR*	Transcriptional regulator

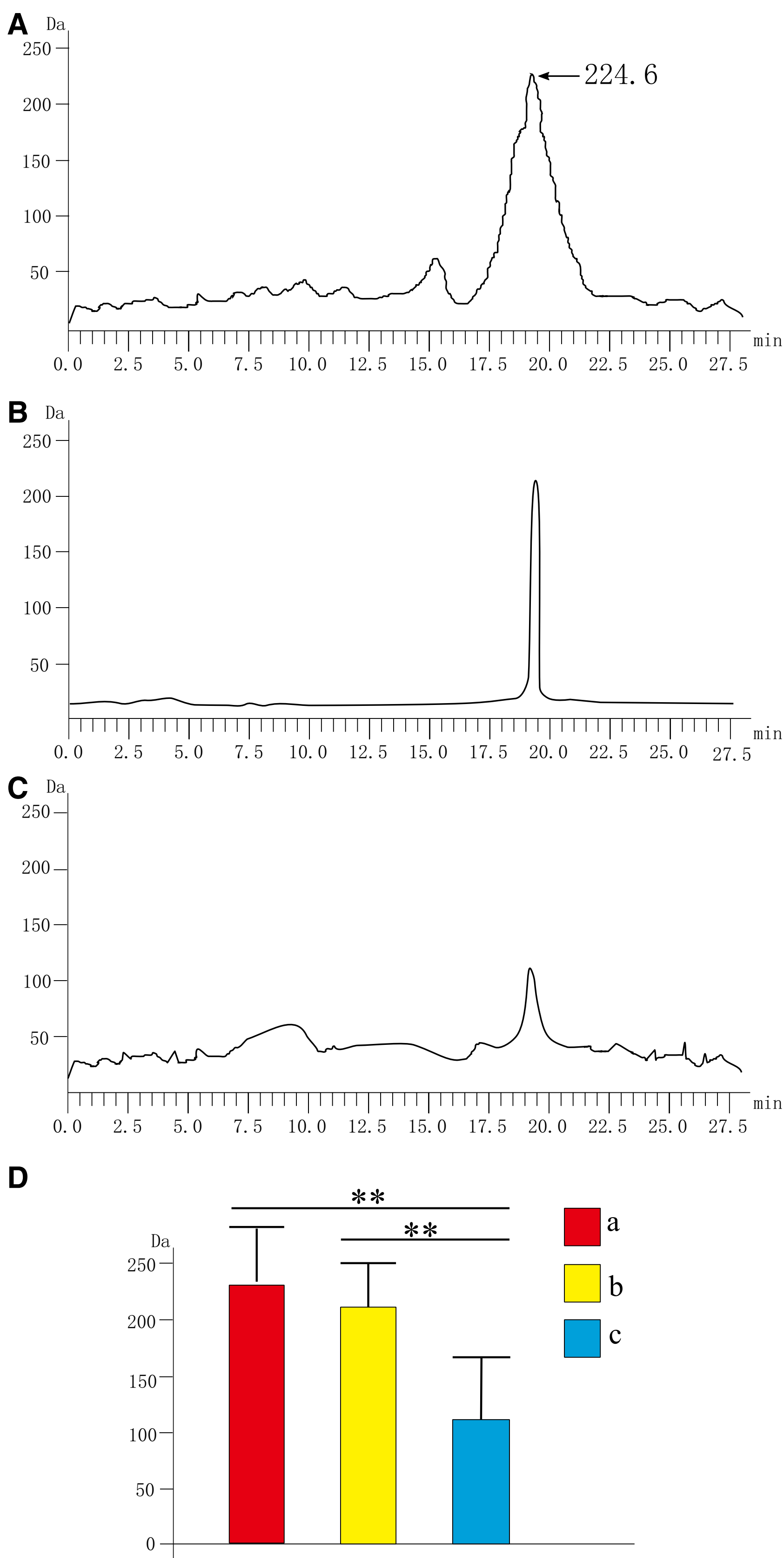

Fig. 1 Comparison of the secondary metabolites from *P. parafulva* WT and *ΔphzF* with purified phenazine-1-carboxylic acid (PCA) using LC-MS. **a** The profiles of secondary metabolites from the WT; **b** the profiles of purified PCA; **c** the profiles of secondary metabolites from *ΔphzF*; **d** the LC-MS peak value comparison among WT, purified PCA and *ΔphzF*. *Da* Dalton, *Min* minutes; ***t* test significance ($P<0.01$)

(Table 2). This pathway includes *phzR,A,G,D,F,H,B,E* genes (Table 2). In these, gene *phzR* is a transcription regulator of PCA biosynthesis [20]. PhzA/B can help to catalyze the formation of the tricycle in PCA biosynthesis pathway [1]. Other genes have also been reported to be important in PCA biosynthesis [12].

To test the hypothesis that the pathway found in our strain confer to the function of PCA biosynthesis, secondary metabolites, which were prepared from *ΔphzF* and WT strains were compared with pure PCA (analytical purity > 98%), which was purchased from Shanghai Qinba Chemical Co., Ltd. using liquid chromatography and mass spectrometry (LC–MS) profiles. *ΔphzF* was made based on the previous report [19]. The secondary metabolites from *P. parafulva* WT and *ΔphzF* were prepared mainly using previous described method [8]. In brief, secondary metabolites were produced from late stationary cultures of *P. parafulva* grown in Mineral Salt Medium (MSM, Composition in g/l: $NaNO_3$ 3 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, KCl 0.5 g, K_2HPO_4 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, Yeast extract 5 g, glycerine 1%, pH 7.0) at 25 °C. 2.5 ml broth was harvested and centrifuged at 10,000 rpm for 10 min under 4 °C. After that, the supernatant was acidified to pH 1.5–2.0 with trifluoroacetic acid (TFA), then extracted into ethyl acetate, and evaporated to dryness [23]. The dried extract was re-dissolved, filtered through Whatman filter paper and loaded onto LC-MS (Agilent 6100, Santa Clara, CA). All the procedures were followed as previously described [5], except the detection wavelength was adjusted to 257 nm and the elution time was adjusted to 19.3 min. Previous report has demonstrated that these parameters can show the maximum peaks for PCA [5]. It is obvious to see from the results that the WT showed a peak as well as the purified PCA around 224 Da, while the peak from the *ΔphzF* was significantly affected (Fig. 1).

In conclusion, the genome of PRS09-11288 contains complete PCA production pathway, which makes it a good candidate as biocontrol agent in crop protection.

Nucleotide Sequence Accession Numbers

This strain has been deposited in CGMCC with accession number CGMCC 1.15632. This genome sequencing project has been deposited at DDBJ/EMBL/GenBank under the accession numbers CP019952. The BioProject designation for this project is PRJNA376819.

Acknowledgements This work was supported by Grants from the Natural Science Fund of China (Grant No. 31521064), and the National Key Technology Research and Development Program (2015BAD01B02). Zhejiang National Natural Science Foundation of China (LY17C010006), Science Foundation of Zhejiang Sci-Tech University (15042168-Y) and Opening foundation of the State Key Laboratory for Diagnosis and Treatment of Infectious Diseases and Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medical College, Zhejiang University (2016KF10).

References

1. Ahuja EG, Janning P, Mentel M, Graebsch A, Breinbauer R, Hiller W, Costisella B, Thomashow LS, Mavrodi DV, Blankenfeldt W (2008) PhzA/B catalyzes the formation of the tricycle in phenazine biosynthesis. J Am Chem Soc 130(50):17053–17061
2. Asaka O, Shoda M (1996) Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. Appl Environ Microbiol 62(11):4081–4085
3. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT (2000) Gene ontology: tool for the unification of biology. Nat Genet 25(1):25–29
4. Bonman J, Khush G, Nelson R (1992) Breeding rice for resistance to pests. Annu Rev Phytopathol 30(1):507–528
5. Denning GM, Iyer SS, Reszka KJ, O'Malley Y, Rasmussen GT, Britigan BE (2003) Phenazine-1-carboxylic acid, a secondary metabolite of *Pseudomonas aeruginosa*, alters expression of immunomodulatory proteins by human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 285(3):L584-L592
6. Ichikawa N, Sasagawa M, Yamamoto M, Komaki H, Yoshida Y, Yamazaki S, Fujita N (2012) DoBISCUIT: a database of secondary metabolite biosynthetic gene clusters. Nucleic Acids Res. https://doi.org/10.1093/nar/gks1177
7. Jacobsen BJ, Backman A (1993) Biological and cultural plant disease controls: alternatives and supplements to chemicals in IPM systems. Plant Dis 77:311–315
8. Jain R, Pandey A (2016) A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. Microbiol Res 190:63–71
9. Jia Y, Correa-Victoria F, Mcclung A, Zhu L, Liu G, Wamishe Y, Xie J, Marchetti M, Pinson S, Rutger J (2007) Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. Plant Dis 91(5):485–489
10. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA (2009) Circos: an information aesthetic for comparative genomics. Genome Res 19(9):1639–1645
11. Li Z, Pinson S, Marchetti M, Stansel J, Park W (1995) Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet 91(2):382–388
12. Liu F, Zhao Y-L, Wang X, Hu H, Peng H, Wang W, Wang J-F, Zhang X (2015) Elucidation of enzymatic mechanism of phenazine biosynthetic protein PhzF using QM/MM and MD simulations. PLoS ONE 10(9):e0139081
13. Liu Q, Zhang Y, Yu N, Bi Z, Zhu A, Zhan X, Wu W, Yu P, Chen D, Cheng S (2015) Genome sequence of *Pseudomonas parafulva* CRS01-1, an antagonistic bacterium isolated from rice field. J Biotechnol 206:89–90
14. Mavrodi DV, Mavrodi OV, Parejko JA, Bonsall RF, Kwak Y-S, Paulitz TC, Thomashow LS, Weller DM (2011) Accumulation of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of dryland cereals. Appl Environ Microbiol. https://doi.org/10.1128/AEM.06784-11
15. Mohd Jaaffar AK, Parejko JA, Paulitz TC, Weller DM, Thomashow LS (2017) Sensitivity of *Rhizoctonia* isolates to phenazine-1-carboxylic acid and biological control by phenazine-producing

Pseudomonas spp. Phytopathology. https://doi.org/10.1094/PHYTO-1007-1016-0257-R

16. Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159(1):73–81
17. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42:D206–D214
18. Parejko JA, Mavrodi DV, Mavrodi OV, Weller DM, Thomashow LS (2013) Taxonomy and distribution of phenazine-producing *Pseudomonas* spp. in the dryland agroecosystem of the Inland Pacific Northwest, United States. Appl Environ Microbiol 79(12):3887–3891
19. Phelan VV, Moree WJ, Aguilar J, Cornett DS, Koumoutsi A, Noble SM, Pogliano K, Guerrero CA, Dorrestein PC (2014) Impact of a transposon insertion in phzF2 on the specialized metabolite production and interkingdom interactions of *Pseudomonas aeruginosa*. J Bacteriol 196(9):1683–1693
20. Pierson L, Keppenne VD, Wood DW (1994) Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30–84 is regulated by PhzR in response to cell density. J Bacteriol 176(13):3966–3974
21. Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28(1):33–36
22. Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. Appl Environ Microbiol 56(4):908–912
23. Wang Y, Newman DK (2008) Redox reactions of phenazine antibiotics with ferric (hydr) oxides and molecular oxygen. Environ Sci Technol 42(7):2380–2386
24. Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W (2015) antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43(W1):W237–W243
25. Xie G-l, Soad A, Swings J, Mew TW (2003) Diversity of Gram negative bacteria antagonistic against major pathogens of rice from rice seed in the tropic environment. J Zhejiang Univ Sci A 4(4):463–468